A peer-reviewed open-access journal

NeoBiota

Advancing research on alien species and biological invasions

NeoBiota 94: 179–203 (2024)
DOI: 10.3897/neobiota.94.121987

Research Article

Exploring the efficacy of predacious diving beetles as potential nature-based solution for combatting the invasive mosquito *Aedes albopictus* (Skuse, 1894)

Adwine Vanslembrouck[1,2], Kevin Scheers[3], Xavier Vermeersch[4], Rens Hendrickx[5], Anna Schneider[1], Jacobus De Witte[1], Isra Deblauwe[1], Wim Van Bortel[1,6], Friederike Reuss[7], Ruth Müller[1,7,8]

1 *Unit Entomology, Institute of Tropical Medicine, Nationalestraat 155, Antwerp, Belgium*
2 *Department of Biology, University of Antwerp, Groenenborgerlaan 171, Antwerp, Belgium*
3 *Unit Freshwater Habitats, Research Institute Nature and Forest, Havenlaan 88, Brussels, Belgium*
4 *Unit Species, Brussels Environment, Havenlaan 86C, Brussels, Belgium*
5 *Department of Leisure, Green areas region of Antwerp, Turnhoutsebaan 232, Deurne, Belgium*
6 *Outbreak Research Team, Institute of Tropical Medicine, Nationalestraat 155, Antwerp, Belgium*
7 *Unit Medical Entomology and Environmental Toxicology, Institute of Occupational Medicine, Social Medicine and Environmental Medicine, Goethe University, Theodor-Stern-Kai 7, Frankfurt am Main, Germany*
8 *Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, Antwerp, Belgium*

Corresponding author: Adwine Vanslembrouck (avanslembrouck@itg.be)

OPEN ACCESS

Academic editor: Sabrina Kumschick
Received: 1 March 2024
Accepted: 3 July 2024
Published: 2 August 2024

Citation: Vanslembrouck A, Scheers K, Vermeersch X, Hendrickx R, Schneider A, De Witte J, Deblauwe I, Van Bortel W, Reuss F, Müller R (2024) Exploring the efficacy of predacious diving beetles as potential nature-based solution for combatting the invasive mosquito *Aedes albopictus* (Skuse, 1894). NeoBiota 94: 179–203. https://doi.org/10.3897/neobiota.94.121987

Copyright: © Adwine Vanslembrouck et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Abstract

The invasive mosquito species *Aedes albopictus* (Skuse, 1894) is rapidly spreading in Europe, posing an increasing threat because of its high vector competence for chikungunya and dengue virus. An integrative and eco-friendly control of these populations is required to prevent mosquito-borne disease outbreaks. Traditionally-used insecticides or other chemical control agents are often expensive, harmful to the environment, strictly controlled or completely banned in several countries. Additionally, insecticide resistance is a potential threat. One possibility for biological control agents is the use of native aquatic beetles as natural predators of mosquitoes to boost *Bacillus thuringiensis israelensis* (Bti) interventions. Thirty predatory aquatic beetle taxa were caught in Belgium and kept at the Institute of Tropical Medicine's insectary to test predation rate and prey choice on *Aedes albopictus* and *Culex pipiens* Linnaeus, 1758. Predation rates suggest at least four efficient dytiscid predators that are known to inhabit small, temporary habitats in Europe. Further experiments on prey choice reveal a clear preference for *Aedes albopictus* over alternative larval prey (*Culex pipiens*, *Daphnia* sp., Chaoboridae). We found a strong ecological overlap of the feeding niche of *A. albopictus* and the hunting zone of dytiscid predators in the benthic layer of small waterbodies. Our findings on the efficacy are very encouraging to further assess the potential of native predacious diving beetles as a biological control agent against the invasive *A. albopictus* in Europe.

Key words: Arbovirus, biodiversity, Dytiscidae, invasion, predation

Introduction

The Asian tiger mosquito *Aedes albopictus* (Skuse, 1894) is an invasive species that rapidly spreads throughout Europe (Sherpa et al. 2019). It poses an increasing threat because of its high vector competence for arboviruses such as chikungunya,

dengue and zika (Martinet et al. 2019; Ryan et al. 2019). Since 2000, an increase in autochthonous infections of dengue and chikungunya virus was observed in Europe (Giunti et al. 2023), for example, 65 autochthonous cases of dengue were recorded in France in 2022 (Gossner et al. 2018; Cochet et al. 2022).

Aedes albopictus females search for blood meals during the day and prefer human hosts in urban areas (Bonizzoni et al. 2013), resulting in potential pathogen transmission and biting nuisance. The establishment of invasive mosquitoes in a yet unaffected area is mainly influenced by climate conditions (Schindler et al. 2015; Ryan et al. 2019), globalisation and urbanisation (Deblauwe et al. 2022b). In addition, these mosquito species are also adapting to climatic changes (Kramer et al. 2020, 2021). At the local scale, interspecific interactions and, foremost, the competition for ecological niches may shape the micro-distribution and abundance of a given *A. albopictus* population (Deblauwe et al. 2015; Müller et al. 2018).

To date, there are no effective vaccines or treatments widely available for dengue and chikungunya virus (Flandes et al. 2023; Thomas 2023; Al-Osaimi et al. 2024). Hence, the prevention of those arboviral infections is primarily based on the control of the mosquito vector (Vontas et al. 2012; Bonizzoni et al. 2013; Abdelnabi et al. 2017). The insecticides used for space spraying, indoor residual spraying or container treatment lead to insecticide resistance, which can rapidly develop in mosquitoes (Su et al. 2019; Pichler et al. 2022; Vereecken et al. 2022). In addition, insecticide-based control efforts over the past decades have not been successful in controlling *Aedes* populations (Achee et al. 2019). In Europe, a biological control method with *Bacillus thuringiensis israelensis* (Bti) is widely used to control mosquito populations (Giunti et al. 2023); however, its activity period is limited (Kroeger et al. 2013; Pauly et al. 2022). In addition, cryptic and domiciliary larval habitats may frequently go unnoticed (Achee et al. 2019). The enrichment of native aquatic biodiversity might be a promising nature-based solution to control discontinuously distributed *A. albopictus* populations. This form of biological control can synergise other control strategies, such as Bti, by including cryptic or domiciliary larval habitats, which often remain undetected or unidentified in other control strategies (Achee et al. 2019; Donald et al. 2020). Additionally, biological vector control generally receives more public acceptance if compared to chemical control (Reuss et al. 2020).

Alternative strategies are mainly focused on adult control and involve *Wolbachia* bacteria (Caputo et al. 2023), sterile insect technique (Balatsos et al. 2024), release of insects carrying a dominant lethal gene (Dobson 2021), attractive toxic sugar baits (Chiu et al. 2024), mass-trapping (Jaffal et al. 2023), spatial repellents, insecticide treated materials (Senapati et al. 2019), antipathogen genetic modifications and lethal ovitraps (Achee et al. 2019; Jones et al. 2021; Ogunlade et al. 2023). For juvenile stages, the use of a new entomopathogenic fungi (Cafarchia et al. 2022) or autodissemination (such as pyriproxyfen) (Pleydell and Bouyer 2019) are proposed alternative strategies (Achee et al. 2019). Biological mosquito control is so far mainly based on predatory copepods, *Toxorhynchites larvae* and fish; however, none of these control agents is currently used in Europe (Baldacchino et al. 2015; Achee et al. 2019).

Biological vector control increases the necessity to identify the most locally effective natural predators of Culicidae, which is especially true for areas recently invaded by *Aedes albopictus* (Younes 2008; Bofill and Yee 2019). The use of native cyclopoid copepods already proved to be a successful method to control first

larval instars of *A. albopictus* (Pauly et al. 2022) and is applied in the Americas, Asia and Oceania (Baldacchino et al. 2015). *Toxorhynchites* species show positive results in the control of *Aedes* species, but they are native to the Tropics with some species found in Asia and North America (Donald et al. 2020; Malla et al. 2023). Moreover, fish such as mosquitofish (*Gambusia* sp.) showed no preference towards mosquito larvae, were introduced far outside its natural distribution range and some species are now considered invasive species (Alcaraz et al. 2008; Jourdan et al. 2021; von der Leyen 2022).

In contrast, many Dytiscidae or predacious diving beetles show a preference to feed on mosquito larvae (Culler and Lamp 2009; Bofill and Yee 2019). The presence of Dytiscidae caused Culicidae populations to decrease significantly (Lundkvist et al. 2003; Chandra et al. 2008; Culler and Lamp 2009; Bofill and Yee 2019). Both larvae and adults of Dytiscidae are considered ubiquitous top predators in lentic systems (Yee 2014), particularly in fishless waters (Larson et al. 2000; Bofill and Yee 2019). Adults of most dytiscid species are capable of active dispersal due to their ability to fly (Bofill and Yee 2019) and many are pioneers occupying freshly-formed waters (Lundkvist et al. 2003; Reyne et al. 2020). They are also found in urban areas (Lundkvist et al. 2002; Liao et al. 2020), which is of paramount importance since urbanisation decreases species diversity and favours *Aedes albopictus* population growth (Perrin et al. 2022).

Dytiscidae are known to migrate, entering a large variety of aquatic habitats and may even have seasonal habitat-shifts or winged migrations (Nilsson and Holmen 1995). Larson et al. (2000) mention that adults of many Dytiscidae are known to disperse readily and are frequently encountered across a wide variety of waterbodies. This also includes artificial habitats as observed by Bameul (1990), Shaverdo et al. (2013) and Nilsson (2024). Fransiscolo (1979) states that isolated individuals can be found anywhere and refers to specimens that were found near and in the sea. Many species reproduce in ephemeral waterbodies, such as small ponds, bogs or ditches with aquatic vegetation. Some even choose small ponds, pits or ditches with little vegetation (Galewski 1971).

Since mosquito larvae are an important prey item for Dytiscidae (Galewski 1971), predacious diving beetles are known to naturally colonise habitats with mosquito larvae, which has been observed by Lundkvist et al. (2003). Especially *Agabus* species seem to prefer smaller waterbodies (Davy-Bowker 2002; Lundkvist et al. 2003).

Onyeka (1983) found eight species of Dytiscidae in 107 litre artificial containers, with 46.3% of 432 specimens testing positive for *Culex pipiens/torrentium* antiserum. Dytiscidae also inhabit tree holes, phytotelmata and water-filled leaves (Kitching and Orr 1996; Miller and Bergsten 2016). Kehl and Dettner (2007) observed them in wells, cattle troughs, swimming pools and rain barrels. Balfour-Browne (1940), Yanoviak (2001) and Nilsson (2024) noted dytiscids in open tub aquaria, paddling pools and plastic cups and pans. Bameul (1990) identified 16 species in an urban pool. James (1965) and Bay (1974) found dytiscids in rock pools preying on *Aedes atropalpus*, which breeds in the same habitats as *A. albopictus* (Kesavaraju et al. 2011; Farajollahi and Price 2013). Young (1954) reported *Laccophilus* sp. in puddles, rain barrels, tin cans and water-filled tyres. Bashir et al. (2017, 2018) observed them with *A. albopictus* larvae in temporary pools and latex collection cups, while Sulaiman and Jeffery (1986) noted Dytiscidae preying on *A. albopictus* larvae.

Until now, research on predation by Dytiscidae focused mainly on their habitat characteristics (Ohba and Ushio 2015), type of prey (Culler and Lamp 2009), preferred larval stage of the prey (Chandra et al. 2008; Younes 2008) and dytiscid stages (larvae and adult) predating on mosquito prey (Bofill and Yee 2019). For example, larval *Acilius sulcatus* collected in India consumed 34 specimens of late instar *Culex quinquefasciatus* in 24 h (Chandra et al. 2008). Though literature supports the statement that predacious diving beetles are effective predators of mosquito larvae, it is unknown which species are the most effective and suitable predators, particularly in areas newly invaded by *Aedes albopictus* (Ohba and Takagi 2010). Some studies in India (Kumar et al. 2014) and Malaysia (Sulaiman and Jeffery 1986) showed positive results using Dytiscidae as a natural predator for *A. albopictus* in laboratory and field conditions. Bashir et al. (2018) proposed to use a dytiscid species as an efficient biological control agent against *A. albopictus* larvae. In general, literature on the topic is mainly focused on Asia and the United States of America. Only very little research has been conducted on whether and which dytiscid species in Europe would be suitable for integrated biological control of mosquitoes, especially for *A. albopictus*. Are all dytiscid species equally suitable as biological control agent against mosquito larvae? Additionally, would aquatic beetles prefer mosquito larvae over other aquatic invertebrates? We here provide research on predatory aquatic beetles for Europe.

We hypothesise that dytiscid species are potentially good biological control agents given that they: 1) show high feeding rate with preference towards Culicidae; 2) are common and widespread throughout Europe and 3) occur in the same region and habitat as *Aedes albopictus* larvae.

In this study, we aimed to assess the feeding preference of 30 predacious diving beetle taxa comparing: 1) mosquito larvae with other aquatic invertebrates and 2) *Aedes albopictus* to *Culex pipiens* larvae. Based on the experimental data, we evaluated whether predacious diving beetles have the potential as a biological control agent against *A. albopictus* and compared the overlap in the field distribution of the most efficient predator species with the points of entry (PoEs) of *A. albopictus* in Belgium.

Methods

Beetle material

A total of 29 species of Dytiscidae and one species of Noteridae were tested. They were collected in semi-permanent pools with a hydrobiological hand net with diameter of 30 cm and a mesh size of 1 mm. Sampling took place in Stekene (51°14'35.5"N, 4°04'11.4"E; Stropersbos), Verrebroek (51°14'44.0"N, 4°14'16.3"E; Haazop) and Kallo (51°15'18.7"N, 4°15'42.4"E; Steenlandpolder) in Belgium on 7 April and 15 November 2021. Species identification was done in the field and nomenclature follows Nilsson (2011) and Nilsson and Hájek (2024). All specimens were maintained in climate chambers (CPS-P530 Climatic Cabinet, RUMED Germany) at the insectary of the Institute of Tropical Medicine and placed at 10 °C with 80% relative humidity and a 16:8 hour light/dark cycle. Adults were maintained together in 200 ml soft water and provided with substrate to hang on to. Larvae were placed separately to avoid cannibalism. Adults and larvae were fed *ad libitum* with frozen chironomid larvae. All specimens were maintained under these conditions

two weeks prior to the experiments to allow acclimatisation. The acclimatisation temperature in the insectary mirrored the mean water temperature during sampling of aquatic beetles. Six mixed water samples from the upper half metre of the water column were taken in Flanders (Belgium) on 13 April (10.5 ± 0.6 °C), 19 October (15.6 ± 0.6 °C) and 1 December 2021 (8.6 ± 0.5 °C) using a WTW Multi 3430 and acid electrode WTW IDS Sentix 940. For the experiments, only healthy, active specimens were retained. Beetles were starved 48 to 72 hours before conducting the experiments. Every specimen was placed in a 100 ml cup with 80 ml of soft water and a stone as substrate at 23 °C. Acclimatisation was allowed for a minimum of one hour prior to every experiment.

Mosquito material

Two mosquito species with different feeding strategies were selected as prey for prey-preference studies with aquatic beetles. *Aedes albopictus* (20AAlb.DE-HU.11) and *Culex pipiens* cf. *molestus* (20CPip.BE-ITMf.6) strains used for the experiments were reared in climate chambers (CPS-P530 Climatic Cabinet, RUMED Germany) at the insectary of the Institute of Tropical Medicine (ITM), Antwerp, Belgium. The *C. pipiens* colony originated from larvae collected in Hove, Belgium (51°09'05.2"N, 4°28'45.2"E) and was reared with overlapping generations for one year at 23.8 °C ± 0.7 °C with 80% relative humidity and a 16:8 hour light/dark cycle. The *A. albopictus* colony derived from a lab strain established at Heidelberg University in 2017 and reared at ITM for six months at 28 °C with 80% relative humidity and a 16:8 hour light/dark cycle. All larvae were fed TetraMin (Tetra, Germany) fish flakes *ad libitum* (Bock et al. 2015).

Feeding experiment

Only third and fourth instar *Aedes albopictus* larvae were used during the experiment and were kept at 20 °C with 80% relative humidity and a 16:8 hour light/dark cycle. To test which predacious diving beetles feed on *A. albopictus* during a feeding experiment, five *A. albopictus* larvae were added to a 100 ml cup hosting a single beetle when starting the experiment. After one hour, the surviving larvae were counted excluding moribund and non-moving larvae. Sometimes the predators started feeding on one larvae and stopped after injuring or killing it. Since this predation is also effective as biological control, we included these moribund and non-moving larvae as dead larvae. All data were obtained in triplicate, except for *Dytiscus marginalis* larvae, *Liopterus haemorrhoidalis*, *Bidessus unistriatus* and *Hydaticus seminiger* with one or two replicates. Feeding rate results of beetles collected in April and in November were compared to assess the effect of seasonality on the feeding behaviour.

Prey choice experiments

To test if predacious diving beetles prefer mosquito larvae over other aquatic invertebrates, a four-choice and a two-choice experiment was performed on a set of effective predators. Third and fourth instar mosquito larvae and freshly bought *Daphnia* sp. and Chaoboridae, kept at 20 °C with 80% relative humidity and a 16:8 hour light/dark cycle, were used during the experiments.

In the four-choice experiment, two larvae of *Aedes albopictus*, *Culex pipiens* and *Chaoborus* sp. and five specimens of *Daphnia* sp. were added to a 100 ml cup hosting one beetle when starting the experiment. After one hour, the surviving prey were counted, excluding moribund and non-moving prey. Five beetle species, that showed to be successful predators in the feeding experiment, were tested in one or two replicates.

A two-choice experiment was performed to test if predacious diving beetles prefer *Aedes albopictus* larvae over *Culex pipiens* larvae. Six dytiscid species that showed to be successful predators in the feeding experiment were tested on their preference for *A. albopictus* over *C. pipiens* larvae in duplicate. This limited number of species and replicates tested was depending on available specimens per dytiscid species. Five larvae of both *A. albopictus* and *C. pipiens* were allotted to a 100 ml cup hosting one beetle when starting the experiment. After one hour, the surviving larvae were counted excluding moribund and non-moving larvae.

Photometric assays

To evaluate the potential bias from seasonal sampling and, hence, probably seasonally varying ecophysiological status of aquatic beetles, that could potentially have an effect on their rate of predation, the content of the energy reserves glycogen and lipid of the collected beetles was quantified. Four dytiscid species (*Agabus bipustulatus*, *A. undulatus*, *Hyphydrus ovatus* and *Laccophilus minutus*) that were sampled in high numbers in both April and November, were analysed in triplicate. The length of elytra and wet weight per specimen was measured prior to the homogenisation in order to allow size normalisation. Per specimen, the total content of glycogen, lipids and proteins was analysed via photometric assays according to Van Handel (1985a), Van Handel (1985b) and Bradford (1976), respectively, as described by Bock et al. (2015). Lipid, glycogen and protein concentrations and weight were divided by elytra length to correct for individual size.

Habitat overlap

The habitat overlap between Dytiscidae and invasive mosquitoes such as *Aedes albopictus* is largely understudied. We observed an influence of separation of entomological disciplines and combined observations of Dytiscidae in invasive *Aedes* habitats in Table 3.

Statistical analysis

The analysis of experimental data and data visualisation was carried out with Prism® (version 9.3.1, GraphPad Software Inc., USA). Statistical significance was defined as $P < 0.05$. The Kolmogorov-Smirnov test and Shapiro-Wilk test were used to test for normality and residuals were plotted to test for homoscedasticity. The feeding rate obtained in triplicate in April and November of five dytiscid species (*Agabus bipustulatus*, *Graptodytes bilineatus*, *Hydroporus angustatus*, *Hygrotus impressopunctatus* and *Laccophilus minutus*) was tested for normality via the Kolmogorov-Smirnov test and verified for homoscedasticity via the homoscedasticity plot. Lipid data were log transformed and glycogen data were sine transformed to meet assumptions of normality. A repeated measures two-way ANOVA was

conducted to verify differences in variation of the feeding rate between both experimental points of time with factors *Species* and *Seasonality*. To merge data from April and November and to compare lipid, protein and glycogen content, a two-way ANOVA was used to test significant differences in variation. Afterwards, the Šídák's multiple comparisons test was conducted to test the species separately.

To assess the overlap between the distribution of predacious diving beetles and the points of entry of *Aedes albopictus*, distribution and presence data were obtained from the *A. albopictus* surveillance programme in Belgium that has been conducted by ITM from 2007 to 2020 (Deblauwe et al. 2022a, 2022b). The Belgian Hydradephaga Database (managed by Scheers, Research Institute Nature and Forest) and Scheers et al. (in press) provided annual information since 1834 on the distribution (exact location or 1 km quadrant) of the dytiscid species. A distribution table with *A. albopictus* presence and presence/absence data of selected beetle species was created resulting in an overlapping distribution table of both Dytiscidae and PoE of *A. albopictus* in Belgium. Presence/absence data of Dytiscidae was given in following classifications: confirmed presence (already been observed), presumed presence (not been observed, but occurrence in the region and suitable habitat present), plausible presence (not been observed, no occurrence in the region, but suitable habitat present) and not present in the region.

A duplicated scoring with variation of expert judgement was performed to rank the top ten predatory beetle species according to our hypothesis that dytiscid species are potentially good biological control agents when they: 1) show high feeding rate with preference towards Culicidae; 2) are common and widespread throughout Europe and 3) occur in the same region as *A. albopictus* larvae. Scoring of the suitability of a given dysticid species as biological control tool against *A. albopictus* was given on ten, including categories such as habitat suitability (small, temporal, ephemeral waters), abundance, dispersal (ability to fly) and distribution, based on Kehl and Dettner (2007), Nilsson and Hájek (2024) and expert judgement.

Results

Rate of predation on *Aedes albopictus* by aquatic beetles

A total of 369 specimens representing 29 predacious diving beetle taxa (Dytiscidae) and one burrowing water beetle species (Noteridae) were collected (Suppl. material 1: table S1). During the feeding experiment, only *Agabus undulatus* consistently consumed all five *Aedes albopictus* larvae in one hour in April. However, the beetles collected in November showed no interest in feeding (Fig. 1). Other *Agabus* species, such as *A. bipustulatus* and *A. nebulosus*, also proved to be very effective predators, consuming on average 83% and 76% of the provided *Aedes albopictus* larvae, respectively. *Rhantus exsoletus* and *Hyphydrus ovatus* consumed 80% and 67%, respectively; however, no *H. ovatus* showed interest in feeding during experiments in November. The Agabinae larvae consumed on average 40% of the *Aedes albopictus* larvae. *Hygrotus impressopunctatus* and *H. parallellogrammus* consumed on average 37% and 53% of the larvae, respectively. *Hydroporus figuratus* fed on average on 47% and *Ilybius quadriguttatus* and *Hydroporus palustris* consumed 40% and 32% of the provided larvae, respectively. *Laccophilus minutus* consumed on average 43% of the larva. All other species (*Acilius sulcatus*, *Clemnius decoratus*, *Colymbetes fuscus*, *Dytiscus marginalis* adults, *Graptodytes bilineatus*,

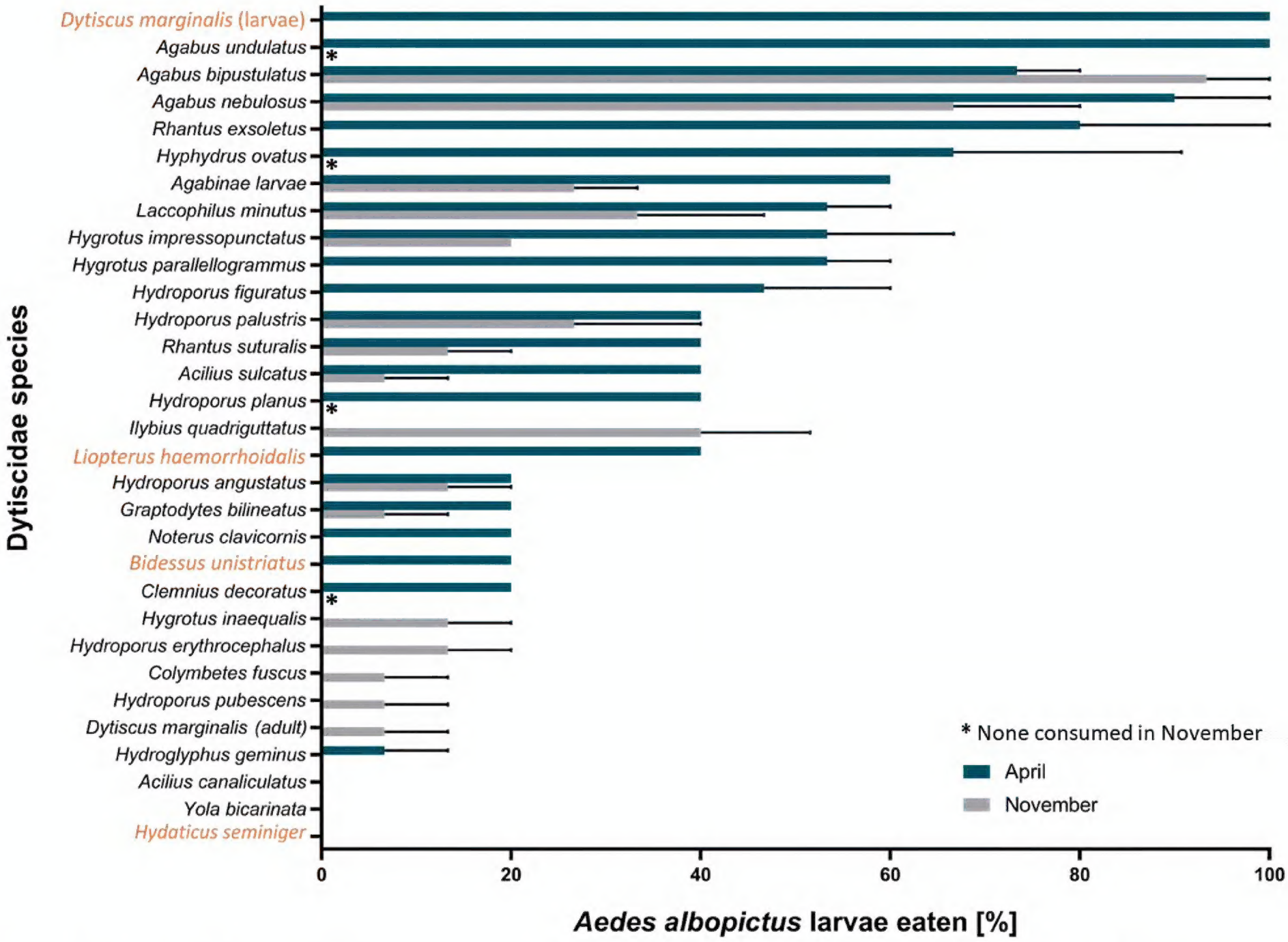

Figure 1. Rate of predation on *Aedes albopictus* larvae by different aquatic beetle species [% larvae eaten per hour]. The percentage of eaten mosquito larvae (n = 5) per dytiscid species during one hour is separately shown for dytiscid specimens collected in the field in either April or November (mean = 3, less replicates for species marked in orange font). * None consumed in November: no predation observed.

Hydroporus angustatus, *H. erythrocephalus*, *H. planus*, *H. pubescens*, *Hydroglyphus geminus*, *Hygrotus inaequalis*, *Noterus clavicornis* and *Rhantus suturalis*) consumed less than 20% of the provided larvae. No predation was observed by *Acilius canaliculatus* nor *Yola bicarinata*. *Dytiscus marginalis* larvae, *Liopterus haemorrhoidalis*, *Bidessus unistriatus* and *Hydaticus seminiger* consumed 100%, 40%, 20% and 0% of the larvae, respectively. These latter species were tested with less than three replicates per species. The following Belgian aquatic beetles were identified as effective predators on *Aedes albopictus*: *Agabus bipustulatus*, *A. nebulosus*, *A. undulatus*, *Hydroporus figuratus*, *Hygrotus impressopunctatus*, *H. parallellogrammus*, *Hyphydrus ovatus*, *Ilybius quadriguttatus*, *Laccophilus minutus* and *Rhantus exsoletus*. These species were included in further experiments.

Normality of the feeding rate data was assumed for both April ($P > 0.1$) and November ($P > 0.1$) datasets. The repeated measures two-way ANOVA indicated that the factor *Beetle species* (73.90%) accounted for most of the variation in the feeding rate and was highly significant ($F = 20.82$; $P < 0.001$). The interaction between the factors *Species x Seasonality* (9.19%) and *Seasonality* (3.34%) were significant ($F = 1.89$; $P = 0.02$ and $F = 7.11$; $P = 0.02$, respectively). Therefore, the seasonal rate of predation was separately shown for each of two sampling months (Fig. 1). Overall, the experimental block *Month* was not a significant factor ($F = 1.89$; $P = 0.17$), but it accounted for 8.87% of total variation. The seasonal predation efficiency

of *Hydroporus angustatus* (P = 0.96), *Graptodytes bilineatus* (P = 0.60), *Laccophilus minutus* (P = 0.22) and *Agabus bipustulatus* (P = 0.22) was not significantly different between the months, but Šídák's multiple comparisons showed that the feeding rate of *Hygrotus impressopunctatus* was significantly different between April and November (P = 0.02), with a higher predation rate in April compared to November.

Prey preference of Dytiscidae

1. Dytiscid preferences towards *Aedes albopictus*, *Culex pipiens*, *Chaoborus* and *Daphnia*

From the five most predatory Dytiscidae, both *Agabus nebulosus* and *A. undulatus* preyed on all four prey choices (Fig. 2a). Four species - *A. nebulosus*, *A. undulatus*, *Laccophilus minutus* and *Rhantus exsoletus* - consumed all provided *Aedes albopictus* larvae. Only one species - *A. nebulosus* - consumed all *Culex pipiens* larvae. *Daphnia* sp. were consumed by *A. nebulosus*, *A. undulatus* and *R. exsoletus* and *Chaoborus* larvae were eaten by *A. nebulosus* and *A. undulatus*.

2. Dytiscid preferences towards *Aedes albopictus* and *Culex pipiens*

The six dytiscid species all preyed on *Aedes albopictus* larvae, for which *Agabus undulatus* ate all five larvae in one hour (Fig. 2b). In contrast, only four species, *A. bipustulatus*, *A. undulatus*, *Hygrotus parallellogrammus* and *Laccophilus minutus* also consumed *Culex pipiens* larvae. For most experiments, the dytiscid specimen started feeding on *Aedes albopictus* larvae and switched to *C. pipiens larvae* when *A. albopictus* larvae were depleted.

The effect of season on dytiscid ecophysiology exemplified by four abundant beetle species

Overall, the weight of the aquatic beetles *Agabus bipustulatus*, *A.undulatus*, *Hyphydrus ovatus* and *Laccophilus minutus* was not significantly different between specimens collected in April or November (Table 1). The energy reserves of aquatic

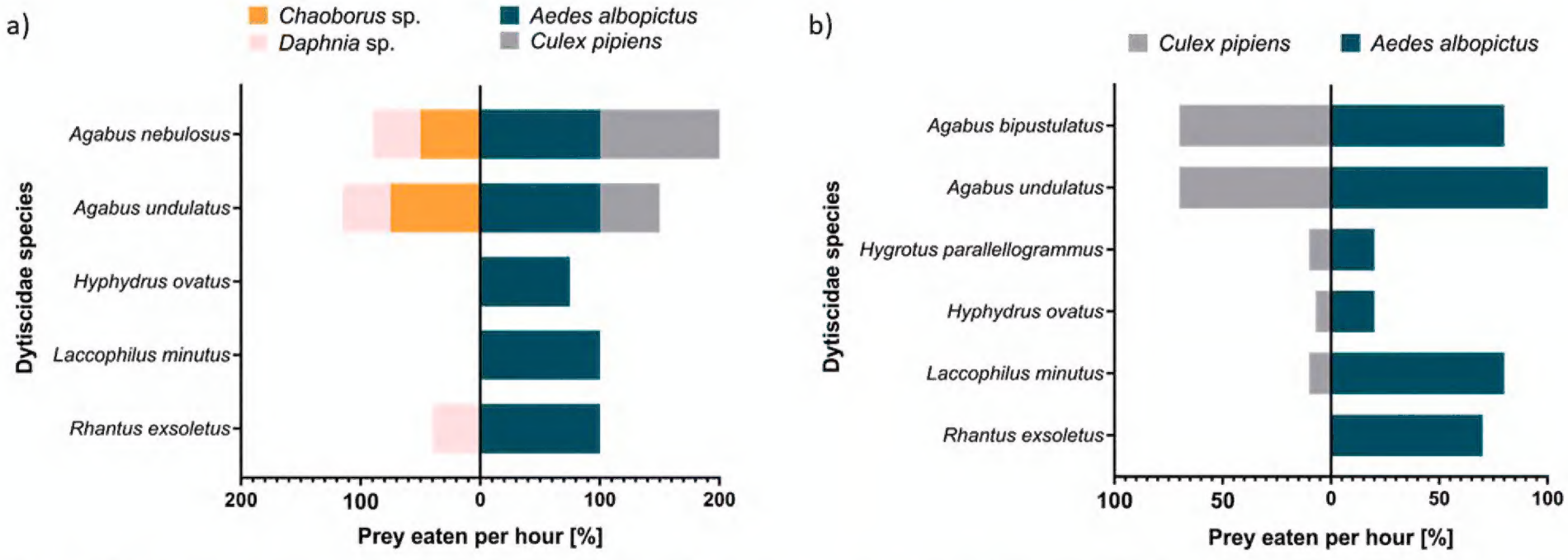

Figure 2. The preferred invertebrate prey of aquatic beetles [% prey eaten per hour] **a** four prey choice experiment offering two larvae of *Aedes albopictus*, *Culex pipiens* and *Chaoborus* sp. each and five *Daphnia* sp. in a volume of 80 ml of water for one hour **b** two prey choice experiment offering five larvae of *A. albopictus* and *C. pipiens* each in a volume of 80 ml of water for one hour.

Table 1. Ecophysiological status of aquatic beetles collected in April and November. The weight [mg], the content of energy reserves and the protein concentration of aquatic beetles [size-corrected lipid, glycogen and protein concentration in µg per adult] are shown for four top predator Dytiscidae.

Weight [mg]	**Mean ± SD April**	**Mean ± SD November**
Agabus undulatus	6.44 ± 0.49	5.75 ± 0.17
Agabus bipustulatus	12.41 ± 0.15	12.56 ± 0.01
Hyphydrus ovatus	4.39 ± 0.56	4.96 ± 0.51
Laccophilus minutus	2.15 ± 0.05	1.75 ± 0.08
Lipids [µg/pupae]	**Mean ± SD April**	**Mean ± SD November**
Agabus undulatus	111.38 ± 14.34	84.19 ± 4.60
Agabus bipustulatus	291.20 ± 49.31	142.35 ± 20.60
Hyphydrus ovatus	80.60 ± 22.58	75.28 ± 4.78
Laccophilus minutus	83.16 ± 11.99	92.38 ± 5.42
Proteins [µg/pupae]	**Mean ± SD April**	**Mean ± SD November**
Agabus undulatus	146.86 ± 7.12	145.33 ± 1.04
Agabus bipustulatus	124.19 ± 3.41	125.05 ± 1.28
Hyphydrus ovatus	25.41 ± 18.33	67.92 ± 19.57
Laccophilus minutus	27.02 ± 4.56	62.89 ± 10.68
Glycogen [µg/pupae]	**Mean ± SD April**	**Mean ± SD November**
Agabus undulatus	22.34 ± 5.70	22.40 ± 4.22
Agabus bipustulatus	31.99 ± 10.39	23.80 ± 6.31
Hyphydrus ovatus	37.86 ± 11.05	118.24 ± 10.11
Laccophilus minutus	3.56 ± 1.05	20.75 ± 2.26

beetles were likewise quite uniform in spring and autumn. However, the lipid content of *A. bipustulatus* was significantly higher in the specimens sampled in April compared to November (P = 0.003) and the glycogen content of *H. ovatus* was much lower if specimens have been sampled in April compared to November. Likewise, the protein content of *L. minutus* and *H. ovatus* was lower in specimens sampled in April compared to November.

Distribution overlap between Dytiscidae and *Aedes albopictus* in Belgium

The known point of entry of *Aedes albopictus* in Belgium were tyre companies, parking lots, a port and a Lucky Bamboo import company (Deblauwe et al. 2022a, 2022b). At least three of the topmost predatory species were expected to be present in the region where *A. albopictus* were already found (Table 2).

Habitat overlap between Dytiscidae and Culicidae

Aedes albopictus has been reported from artificial habitats such as pots, buckets, manhole/scupper and rain barrels with a typical water volume ranging from less than one litre up to 200 litres (Graziosi et al. 2020), which are similar habitats observed for Dytiscidae by Young (1954), Onyeka (1983), Sulaiman and Jeffery (1986) and Bashir et al. (2018). Since the habitat overlap between Dytiscidae and invasive mosquitoes is largely understudied, observations of Dytiscidae in

Table 2. Distribution overlap between Dytiscidae within 5 km from a points of entry (PoE) of *Aedes albopictus*.

Selected beetle species	Points of Entry of *Aedes albopictus*										Points of Entry of *Aedes albopictus*		
	AB	AT	BA	E0	E12	E2	E5	E6	EB	PA1			
Agabus bipustulatus											**Tire companies**	AB	Kallo
Agabus nebulosus												AT	Vrasene
Agabus undulatus												BA	Frameries
Hydroporus figuratus											**Parking lots**	E0	Sprimont
Hygrotus impressopunctatus												E12	Eghezée
Hygrotus parallellogrammus												E2	Messancy
Hyphydrus ovatus												E5	Wanlin
Ilybius quadriguttatus												E6	Kortrijk
Laccophilus minutus											**Lucky Bamboo**	EB	Lochristi
Rhantus exsoletus											**Port**	PA1	Antwerp

Orange: confirmed presence within 5 km (since 2010); blue: presence within 5 km not confirmed but presumed; rose: presence within 5 km not confirmed but plausible; gray: not present in the region.

artificial habitats that were made or verified by the authors were added to Table 3. These observations include plastic trays, plastic foil, buckets, bird baths, ornamental water features, cattle drinking troughs, rain barrels, shallow puddles, garden ponds and disused swimming pools, ranging from less than one litre up to 250 litres. These observations were made in small city gardens, urban parks, suburban gardens, agricultural areas, heathland, nature reserves and forests in Belgium.

Ecological portfolio of the topmost predatory dytiscid species

Agabus bipustulatus has the highest total scoring because of its wide habitat preference, abundance, excellent dispersal ability (Kehl and Dettner 2007) and wide distribution throughout Europe (Nilsson and Hájek 2024) (Table 4). *Agabus nebulosus*, *Laccophilus minutus* and *Hygrotus impressopunctatus* are also suitable species. *Agabus undulatus*, *Hyphydrus ovatus* and *Hydroporus figuratus* were excluded due to their reduced flight ability (Kehl and Dettner 2007). *Ilybius quadriguttatus* was excluded due to its preference for well-vegetated semi-permanent lentic habitats and *Hygrotus parallellogrammus* and *Rhantus exsoletus* were excluded, based on both habitat preference (coastal habitat and vegetated permanent waterbodies, respectively) and distribution (Nilsson and Holmen 1995; Nilsson and Hájek 2024).

Discussion

This study is a first step to understand the value of the use of native Dytiscidae and Noteridae species as a biological *Aedes albopictus* control tool. Firstly, a high feeding rate on *A. albopictus* has been observed in several dytiscid species. Based on Kehl and Dettner (2007), Nilsson and Hájek (2024) and expert judgement, these species resulted in a top ten of best predators for *A. albopictus* larvae. Two prey-choice experiments carried out with a selection of the top ten predators revealed a clear prey preference towards mosquito larvae and especially towards *A. albopictus* larvae. For Belgium, a significant distribution overlap was detected for three efficient

Table 3. Dytiscidae found or verified in small and artificial habitats by the authors in Belgium, overlapping with habitats of *Aedes albopictus*.

Water volume (L)	Larval habitat	Dytiscidae species	Number of individuals	Stage
<1	plastic tray	*Agabus bipustulatus*	1	Adult
4	puddle in piece of plastic foil	*Agabus bipustulatus*	1	Adult
5	bucket	*Hydroporus dorsalis*	1	Adult
		Hydroporus planus	4	
		Hydroporus tesselatus	2	
		Hydroporus pubescens	1	
		Rhantus suturalis	1	
10	small steel bird bath	*Agabus bipustulatus*	1	Adult
		Hydroglyphus geminus	5	
		Hydroporus planus	2	
15	display table for pond plants	*Hydroglyphus geminus*	1	Adult
	small concrete bird bath	*Hydroporus pubescens*	2	Adult
20	disused metal cattle drinking trough	*Agabus bipustulatus*	1	Adult
		Hygrotus inaequalis	4	
	small wooden ornamental water feature	*Hydroporus planus*	1	Adult
30	disused prefab plastic water feature	*Agabus bipustulatus*	9	Adult
		Hydroporus nigrita	3	
		Hydroporus tesselatus	1	
40	disused prefab plastic water feature	*Agabus bipustulatus*	10	Adult
		Hydroporus nigrita	10	
		Ilybius chalconatus	1	
50	cattle watering basin	*Agabus bipustulatus*	21	Adult
		Hydroglyphus geminus	2	
		Hydroporus planus	3	
		Hydroporus pubescens	3	
	plastic ornamental water feature	*Agabus bipustulatus*	>10	Adult
60	concrete water feature	*Hydroporus tesselatus*	1	Adult
90	rain barrel	*Agabus bipustulatus*	1	Adult
		Dytiscus marginalis	1	
	cattle drinking bucket	*Rhantus suturalis*	1	Adult
100	metal cattle drinking trough	*Agabus bipustulatus*	30	Larvae
		Hydroporus discretus	2	Adult
	plastic drinking container for cattle	*Agabus bipustulatus*	3	Adult
		Hydroporus planus	6	
		Rhantus suturalis	2	
	shallow puddle on concrete slab at construction site	*Agabus nebulosus*	1	Adult
		Hydroglyphus geminus	>10	
		Hydroporus planus	1	
		Rhantus suturalis	3	
150	plastic drinking container for cattle	*Agabus uliginosus*	1	Adult
	shallow puddle on concrete slab at construction site	*Agabus bipustulatus*	1	Adult
		Colymbetes fuscus	1	
		Hydroglyphus geminus	>10	
		Hydroporus palustris	1	
		Hydroporus planus	>10	
		Rhantus suturalis	2	
220	rain barrel	*Acilius sulcatus*	1	Larvae
250	metal cattle drinking trough	*Agabus bipustulatus*	1	Adult

Table 4. Ecological portfolio of the top ten dytiscid predators for *Aedes albopictus* based on Kehl and Dettner (2007), Nilsson and Hájek (2024) and expert judgement.

Habitat	To what extent does this species occur in small ephemeral waterbodies?				
Common	Is this species common?				
Dispersal	How is this species' ability to fly?				
Distribution	To what extent does this species occur in Europe?				
Selected beetle species	**Habitat**	**Common**	**Dispersal**	**Distribution**	**Total scoring [%]**
Agabus bipustulatus	7.5	10.0	10.0	10.0	93.75
Agabus nebulosus	6.0	8.0	9.5	9.5	82.50
Laccophilus minutus	3.5	9.5	10.0	10.0	82.50
Hygrotus impressopunctatus	3.5	8.0	8.5	8.5	71.25
Rhantus exsoletus	2.5	5.5	10.0	8.5	66.25
Ilybius quadriguttatus	1.0	7.5	10.0	6.5	62.50
Hyphydrus ovatus	1.0	9.5	1.0	8.5	50.00
Hygrotus parallellogrammus	1.0	1.5	8.0	7.5	45.00
Hydroporus figuratus	1.0	5.0	3.5	6.5	40.00
Agabus undulatus	1.0	2.0	1.0	4.5	21.25

predator species (*Agabus bipustulatus*, *A. nebulosus* and *Laccophilus minutus*) and ten points of entry where *Aedes albopictus* has been introduced in Belgium between 2007 and 2020 (Deblauwe et al. 2022b). The results are very encouraging to further explore the use of Dytiscidae as a potential biological control agent against *Aedes albopictus*.

The obtained results showed evidence for efficacy of predacious diving beetles to predate on *Aedes albopictus* larvae. Following our observations, of the topmost predatory dysticid species are *Agabus undulatus*, *A. bipustulatus*, *A. nebulosus*, *Rhantus exsoletus*, *Hyphydrus ovatus*, *Laccophilus minutus*, *Hygrotus impressopunctatus*, *H. parallellogrammus*, *Hydroporus figuratus* and *Ilybius quadriguttatus*. The tested beetle larvae proved to be good predators, which is in line with Chandra et al. (2008), Culler and Lamp (2009) and Bofill and Yee (2019). However, dytiscid larvae were not included in the topmost predator list, since the active dispersal of the predators is necessary for biological control. Adults are found in a larger variety of waterbodies, while dytiscid larvae are generally ecologically more specialised than adults (Galewski 1971). It is important to note that Dytiscidae show different types of feeding behaviour in their life stages. Larvae can inject digestive protease enzymes and feed on the prey's body contents (Yee 2014). In the majority of dytiscids, the first larval stage has shorter mandibles and feeds mostly on planktonic Crustacea, but older larval stages have longer mandibles and hunt insect larvae such as Culicidae (Galewski 1973). Especially larvae from the genera *Agabus*, *Colymbetes*, *Hydroporus* and *Rhantus* are known to feed mainly on mosquitoes (Galewski 1971). Adults have chewing mouthparts and are thus gape-limited (Yee 2014); however, they will attack any prey they can overtake (Galewski 1971).

The efficient predation on mosquito larvae by *Agabus* species are in line with Ohba and Takagi (2010), where they found a predation rate on fourth instar *Culex tritaeniorhynchus* of 100% and 55% for *Agabus conspicuous* and *A. japonicus*, respectively. The feeding rate for *Hyphydrus ovatus* is comparable to the observation in Ohba and Takagi (2010), with a 44% predation rate for *H. japonicus* on *Culex tritaeniorhynchus*. A similar predation rate was reported for *Laccophilus minutus* on

C. pipiens and *L. difficilis* on *C. tritaeniorhynchus* of 43% and 39%, respectively. Evidence for predation on *Aedes atropalpus* was found for *Laccophilus* sp. (James 1965). The predation rate for *Hydroglyphus geminus* is in line with a 16% feeding rate in *H. japonicus*. In contrast to current observations, *Rhantus suturalis* had a feeding rate of 99% on *C. tritaeniorhynchus* (Ohba and Takagi 2010).

The prey choice experiments showed a clear feeding preference towards Culicidae, specifically to *Aedes albopictus*. When *A. albopictus* was depleted, a switchover to *Culex pipiens* was observed several times, which is in line with Culler and Lamp (2009) and Ohba and Ushio (2015). *Aedes albopictus* was presumably preferred due to its foraging behaviour, since its larvae tend to feed in the benthic zone in a flexing behaviour. In contrast, *C. pipiens* filter-feeds near the water surface and has a motionless behaviour to avoid predation (Yee et al. 2004; Ohba and Ushio 2015). Three videos demonstrating this behaviour are added as supplementary material (Suppl. materials 2–4). Predacious diving beetles tend to hunt their prey in the benthic zone (Lundkvist et al. 2003); hence, they encounter more *A. albopictus* larvae. Chances are high for introduced *A. albopictus* to encounter a suitable predator, since the distribution of these predacious diving beetles largely overlap with at least two species present in the known PoE of *A. albopictus* in Belgium. Some dytiscids occupy similar habitats as *A. albopictus* (Table 3) and are active all year round, except when freezing (Foster and Friday 2011).

In addition to *Aedes albopictus*, there are also two other invasive mosquitoes in Europe, i.e. *A. japonicus* (Theobald, 1901) and *A. koreicus* (Edwards, 1917) (Deblauwe et al. 2022b). Both species share a similar larval foraging behaviour as *A. albopictus* and, therefore, similar results in predation by dytiscids are expected. These species and especially *A. japonicus*, often occur in more natural habitats in their non-native range (Smitz et al. 2021; Deblauwe et al. 2022b) and, therefore, have an even larger overlap in habitat preference with native Dytiscidae. Most probably the foraging behaviour of these mosquito larvae dictates in large part the suitability of aquatic beetles as native predators.

Coinciding with a higher feeding rate in November, lipid concentration was much lower in November compared to April for *Agabus bipustulatus*, which may indicate a shortage of lipids before winter (Arrese and Soulages 2010). *Agabus bipustulatus* is known to be active during winter (Classen and Dettner 1983) and to hunt underneath ice (pers. observ. K. Scheers). For *A. undulatus*, no differences in weight, lipid, protein and glycogen content were found, which coincides with their disinterest to feed in November. This storage in energy and reduced feeding behaviour could indicate an overwintering strategy in the soil, which is also observed for *A. paludosus* (Classen and Dettner 1983). Average glycogen and protein concentration was doubled in November compared to April for both *Laccophilus minutus* and *Hyphydrus ovatus*. However, the difference was not significant. This observation is in line with their active behaviour in winter, since glycogen is synthesised to sugar alcohols as an adaptation to cold (Arrese and Soulages 2010). Further studies on the feeding behaviour of Dytiscidae should include seasonal effect and age of the used specimens to avoid seasonal bias.

From a European biological control perspective, *Agabus bipustulatus* seems to be the most suitable predator to reduce mosquito larvae, especially *Aedes albopictus* larvae. The species is known to occur in artificial containers (Onyeka 1983; Reyne et al. 2020) and temporary pools (Eyre et al. 1986), has a high dispersal rate (Kehl and Dettner 2007), has a broad habitat spectrum and is very common throughout

Europe (Nilsson and Holmen 1995). In addition, *Aedes cantans* and *Culex pipiens/torrentium* antiserum was found in gut smears of field-caught adult and larval *Agabus bipustulatus* (Service 1977; Onyeka 1983), confirming its suitability as a biological predator against mosquito larvae. Further laboratory and field research on predation of mosquito larvae by *A. nebulosus*, *Hygrotus impressopunctatus* and *Laccophilus minutus* is required to assess whether these species are suitable biological control agents, since these species are largely understudied.

Currently, there is no literature available on the release of diving beetles in Europe. In terms of ecosystem impact, the introduction of additional native diving beetles as proposed in the present study could potentially compensate for loss of biodiversity, especially in biodiversity-poor areas commonly associated with *Aedes albopictus* infestations (Perrin et al. 2022; Giunti et al. 2023). Competition between Dytiscidae will be minimal, since adults are capable of active dispersal (Kehl and Dettner 2007); however, there is potential for competition and cannibalism amongst diving beetle larvae (Inoda and Kamimura 2004). Evaluating these interactions will be essential for predicting the success and sustainability of using Dytiscidae as biocontrol agents.

When considering the introduction of diving beetles as biological control agents against *A. albopictus*, it is crucial to account for several important non-target effects. Although the present study includes various prey species, such as Chaoboridae and *Daphnia* sp., further extensive field studies are necessary to include all naturally occurring prey and predators. This broader assessment will ensure a comprehensive understanding of the ecological impacts. Historical evidence indicates that generalist and specifically non-native predators often proved to become problematic. For instance, the introduction of the cane toad in Australia (Shine et al. 2020) and the mosquitofish in various regions (Jourdan et al. 2021) led to significant ecological disruptions due to their generalist feeding habits and competition with native species. In contrast, the use of native species, such as copepods (Giunti et al. 2023) and *Toxorhynchites* mosquitoes (Malla et al. 2023), could reduce negative ecological impacts.

This form of biological control may synergise with another biological control method that is already widely used in Europe: the use of Bti. This form of integrated vector control may work well with predacious beetles, since they are not affected by Bti (Shaalan and Canyon 2009) and studies showed that predator cues even increased mortality on *Culex pipiens* and *C. quinquefasciatus* when combined with Bti (Op De Beeck et al. 2016; Delnat et al. 2020. In addition, the dytiscids can invade cryptic or domiciliary breeding sites, which often remain undetected or unidentified during Bti application (Achee et al. 2019; Donald et al. 2020).

Rearing of Dytiscidae remains a major challenge due to their high rate of food consumption and their intrinsic cannibalistic behaviour (Inoda and Kamimura 2004; Inoda and Kitano 2013). Dytiscid larvae feed on dissolved body fluids, resulting in food refusal and irreversibly spoiling the water (Inoda and Kamimura 2004). A first solution could be deploying the larval stages in close proximity with *Aedes albopictus* populations to avoid the laboratory rearing to adulthood. Another possibility would be using the rearing technique developed for mass rearing of juvenile lobsters (*Homarus gammarus*), which could resolve the rearing problem by keeping juveniles separately in clean water with a high quality food source (Schmalenbach et al. 2009). Moreover, the terrestrial pupation stage of water beetles in soil is an additional element that is absent in many other reared aquatic

animals and which makes rearing on larger scale more difficult. Once resolved, mass-reared Dytiscidae could be released in *A. albopictus* infested areas to aid population control. In addition, species such as *Agabus bipustulatus* have been found to live over two years, meaning a frequent release of these species would be unnecessary (Davy-Bowker 2002).

The results also underline the suitability and possibly important role of native predators in the ongoing battle against invasive species, such as the vectors of mosquito-borne diseases. Good habitat quality and high native predatory insect densities can prevent the establishment of invasive mosquitos (Juliano and Lounibos 2005). Moreover, high species richness of these native predators can assure the presence of suitable species which co-occur in the same habitat and have a compatible foraging behaviour. Restoration of degraded aquatic habitats is an important issue, however, often limited to larger bodies of water and lotic environments. In the light of the management of invasive mosquito species, also small fishless temporary waterbodies should be taken into account in nature management and restoration projects (Liao et al. 2020). Though the habitat overlap between *Aedes albopictus* and Dytiscidae is not 100% secure, we do observe an influence of separation of entomological disciplines: Culicidae specialists aim to find *A. albopictus* for monitoring purposes and do not record other species found in these habitats, while Dytiscidae specialists focus on natural habitats and rarely search specimens in tyres or artificial containers in gardens. From this perspective, it would be beneficial to underline the importance of recording these findings.

During this study, a potential bias was created since the beetles were fed solely under laboratory conditions and, therefore, forced to feed on selected prey, which might differ from their natural preference. They were fed with *Culex pipiens* and *A. albopictus* larvae, both accounting for the diverse feeding strategies of mosquitoes. In addition, *Chaoborus* sp. larvae were included since they resemble mosquito larvae and are very common in lentic waters. *Daphnia* sp., generally found in ephemeral ponds and small waterbodies, were added to include a completely different type of prey. Chironomidae were not included in this study since they prefer waters with sediment, which was beyond the scope of this study. Therefore, it is assumed that prey choices most likely available were added to the study and, hence, reducing the influence of bias.

Conclusion

We provide some evidence on the efficacy of Dytiscidae to predate on *Aedes albopictus* larvae. In total, the feeding rate of thirty aquatic beetle taxa on *A. albopictus* larvae were tested, accounting for almost 25% of the total Dytiscidae diversity in Belgium and one out of two Noteridae species present (Scheers et al., in press). When giving Dytiscidae the choice to feed on taxa other than mosquitoes, such as Chaoboridae and Cladocera, the tested species still preferred Culicidae larvae, with a clear preference towards *A. albopictus* over *Culex pipiens*. The distribution of best-predating species of Dytiscidae in our tests shows a clear overlap with the PoE where *A. albopictus* has been found in Belgium in recent years. Several species which are widespread in Europe, such as *Agabus bipustulatus*, *A. nebulosus*, *Hygrotus impressopunctatus* and *Laccophilus minutus* also show a high predation rate and clear preference for *Aedes albopictus*. *Agabus bipustulatus* seems to be the most

suitable predator for *Aedes albopictus*, since this species is known to occur in artificial containers and temporary pools, has a high dispersal rate and is very common throughout Europe. Given these promising results for a nature-based solution, the use of Dytiscidae as a biological control agent against *A. albopictus* should be further investigated.

Acknowledgements

We would like to thank Marre van de Ven and Chaymae Kadi for their contribution to the research. We are grateful to the laboratory and technical staff at the Institute of Tropical Medicine Antwerp, Belgium, especially Luka Wouters, Leen Denis and Karen Jennes for their support of lab experiments. We would like to thank Tom Vermeire for his additional data on Dytiscidae in artificial habitats in Belgium. We also thank Dr. Juliane Hartke for her help during the experiments and Prof. Konrad Dettner for his insights into the seasonal effects. We thank Prof. Norbert Becker for providing us with the *Aedes albopictus* colony.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research was funded through the 2018–2019 BiodivERsA joint call for research proposals, under the BiodivERsA3 ERA-Net COFUND programme (project DiMoC – Diversity Components of Mosquito-borne Diseases under Climate Change) and with the funding organisation FWO G0G2319N. The research was also funded through project BIOZ by the Federal Ministry of Health of Germany under the research network programme "Nationales Forschungsnetz Zoonotische Infektionskrankheiten" 2521NIK401 and the MEMO and MEMO+2020 projects (2017–2020) by the Flemish, Walloon and Brussels regional governments and the Federal Public Service (FPS) Public Health, Food Chain Safety and Environment in the context of the National Environment and Health Action Plan (NEHAP) (Belgium). ITM's outbreak research team is supported by the Department of Economy, Science and Innovation of the Flemish government, Belgium. The insectaries at ITM are partially funded through the Department of Economy, Science and Innovation (EWI) of the Flemish Government.

Author contributions

AV: Conceptualisation, Data Curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualisation, Writing – original draft. KS: Conceptualisation, Data Curation, Resources, Writing – review and editing. XV: Resources, Visualisation, Writing – review and editing. RH: Conceptualisation, Resources, Writing – review and editing. AS: Resources, Writing – review and editing. JDW: Resources, Writing – review and editing. ID: Resources, Writing – review and editing. WVB: Resources, Writing – review and editing. FR: Conceptualisation, Methodology, Writing – review and editing. RM: Conceptualisation, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – review and editing.

Author ORCIDs

Adwine Vanslembrouck https://orcid.org/0000-0002-7304-2493
Kevin Scheers https://orcid.org/0000-0002-4756-4247
Xavier Vermeersch https://orcid.org/0000-0003-1752-8226
Anna Schneider https://orcid.org/0000-0001-9449-5902
Isra Deblauwe https://orcid.org/0000-0001-7268-8965
Wim Van Bortel https://orcid.org/0000-0002-6644-518X
Friederike Reuss https://orcid.org/0009-0008-6967-179X
Ruth Müller https://orcid.org/0000-0003-3909-3876

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Abdelnabi R, Neyts J, Delang L (2017) Chikungunya virus infections: Time to act, time to treat. Current Opinion in Virology 24: 25–30. https://doi.org/10.1016/j.coviro.2017.03.016

Achee NL, Grieco JP, Vatandoost H, Seixas G, Pinto J, Ching-Ng L, Martins AJ, Juntarajumnong W, Corbel V, Gouagna C, David JP, Logan JG, Orsborne J, Marois E, Devine GJ, Vontas J (2019) Alternative strategies for mosquito-borne arbovirus control. PLoS Neglected Tropical Diseases 13(1): e0006822. https://doi.org/10.1371/journal.pntd.0006822

Al-Osaimi HM, Kanan M, Marghlani L, Al-Rowaili B, Albalawi R, Saad A, Alasmari S, Althobaiti K, Alhulaili Z, Alanzi A, Alqarni R, Alsofiyani R, Shrwani R (2024) A systematic review on malaria and dengue vaccines for the effective management of these mosquito borne diseases: Improving public health. Human Vaccines & Immunotherapeutics 20(1): 2337985. https://doi.org/10.1080/21645515.2024.2337985

Alcaraz C, Bisazza A, Garcia-Berthou E (2008) Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. Oecologia 155(1): 205–213. https://doi.org/10.1007/s00442-007-0899-4

Arrese EL, Soulages JL (2010) Insect Fat Body: Energy, Metabolism, and Regulation. Annual Review of Entomology 55(1): 207–225. https://doi.org/10.1146/annurev-ento-112408-085356

Balatsos G, Karras V, Puggioli A, Balestrino F, Bellini R, Papachristos DP, Milonas PG, Papadopoulos NT, Malfacini M, Carrieri M, Kapranas A, Mamai W, Mastronikolos G, Lytra I, Bouyer J, Michaelakis A (2024) Sterile Insect Technique (SIT) field trial targeting the suppression of *Aedes albopictus* in Greece. Parasite 31: 17. https://doi.org/10.1051/parasite/2024020

Baldacchino F, Caputo B, Chandre F, Drago A, della Torre A, Montarsi F, Rizzoli A (2015) Control methods against invasive *Aedes* mosquitoes in Europe: A review. Pest Management Science 71(11): 1471–1485. https://doi.org/10.1002/ps.4044

Balfour-Browne F (1940) British Water Beetles (Vol. I). Ray Society, London.

Bameul F (1990) Observations sur des coléoptères aquatiques récoltés dans une piscine publique de la ville de Bordeaux (Coleoptera Haliplidae, Dytiscidae, Hydrophilidae, Dryopidae). Bulletin de la société linnéenne de Bordeaux 18: 41–52.

Bashir A, Kumar NP, Khan AB (2017) Climatic factors influences the ecology of the *Platynectes* sp. nov. Predator to the dengue and chikungunya vectors in the rubber plantations of Kerala, India. International Journal of Zoology Studies 2(5): 68–77.

Bashir A, Kumar NP, Khan AB (2018) Description of a new species, *Platynectes sahyadriensis* (Coleoptera: Dytiscidae), predatory to *Aedes albopictus* (Diptera: Culicidae) from the foothills of West-

ern Ghats, Kerala, India. Biomedical Journal of Scientific & Technical Research 2(3): 2673–2678. https://doi.org/10.26717/BJSTR.2018.02.000767

Bay EC (1974) Predator-prey relationships among aquatic insects. Annual Review of Entomology 19(1): 441–453. https://doi.org/10.1146/annurev.en.19.010174.002301

Bock F, Kuch U, Pfenninger M, Müller R (2015) Standardized Laboratory Feeding of Larval *Aedes japonicus japonicus* (Diptera: Culicidae). Journal of Insect Science 15(1): 144. https://doi.org/10.1093/jisesa/iev126

Bofill CE, Yee DA (2019) An army of one: Predacious diving beetle life history stages affect interactions with shared mosquito prey. Hydrobiologia 827(1): 201–209. https://doi.org/10.1007/s10750-018-3765-y

Bonizzoni M, Gasperi G, Chen X, James AA (2013) The invasive mosquito species *Aedes albopictus*: Current knowledge and future perspectives. Trends in Parasitology 29(9): 460–468. https://doi.org/10.1016/j.pt.2013.07.003

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1–2): 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cafarchia C, Pellegrino R, Romano V, Friuli M, Demitri C, Pombi M, Benelli G, Otranto D (2022) Delivery and effectiveness of entomopathogenic fungi for mosquito and tick control: Current knowledge and research challenges. Acta Tropica 234: 106627. https://doi.org/10.1016/j.actatropica.2022.106627

Caputo B, Moretti R, Virgillito C, Manica M, Lampazzi E, Lombardi G, Serini P, Pichler V, Beebe NW, Della Torre A, Calvitti M (2023) A bacterium against the tiger: Further evidence of the potential of noninundative releases of males with manipulated *Wolbachia* infection in reducing fertility of *Aedes albopictus* field populations in Italy. Pest Management Science 79(9): 3167–3176. https://doi.org/10.1002/ps.7495

Chandra G, Mandal SK, Ghosh AK, Das D, Banerjee SS, Chakraborty S (2008) Biocontrol of larval mosquitoes by *Acilius sulcatus* (Coleoptera: Dytiscidae). BMC Infectious Diseases 8(1): 138. https://doi.org/10.1186/1471-2334-8-138

Chiu MC, Neoh KB, Hwang SY (2024) The effect of attractive toxic sugar bait on the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae) in community farms in Northern Taiwan. Acta Tropica 250: 107102. https://doi.org/10.1016/j.actatropica.2023.107102

Classen R, Dettner K (1983) Pygidial Defensive Titer and Population-Structure of *Agabus bipustulatus* L and *Agabus paludosus* F (Coleoptera, Dytiscidae). Journal of Chemical Ecology 9(2): 201–209. https://doi.org/10.1007/BF00988037

Cochet A, Calba C, Jourdain F, Grard G, Durand GA, Guinard A, Noel H, Paty MC, Franke F (2022) Autochthonous dengue in mainland France, 2022: Geographical extension and incidence increase. Eurosurveillance 27(44). https://doi.org/10.2807/1560-7917.ES.2022.27.44.2200818

Culler LE, Lamp WO (2009) Selective predation by larval *Agabus* (Coleoptera: Dytiscidae) on mosquitoes: support for conservation-based mosquito suppression in constructed wetlands. Freshwater Biology 54(9): 2003–2014. https://doi.org/10.1111/j.1365-2427.2009.02230.x

Davy-Bowker J (2002) A mark and recapture study of water beetles (Coleoptera: Dytiscidae) in a group of semi-permanent and temporary ponds. Aquatic Ecology 36(3): 435–446. https://doi.org/10.1023/A:1016550127986

Deblauwe I, Demeulemeester J, De Witte J, Hendy A, Sohier C, Madder M (2015) Increased detection of *Aedes albopictus* in Belgium: No overwintering yet, but an intervention strategy is still lacking. Parasitology Research 114(9): 3469–3477. https://doi.org/10.1007/s00436-015-4575-z

Deblauwe I, Brosens D, De Wolf K, Smitz N, Vanslembrouck A, Schneider A, De Witte J, Verle I, Dekoninck W, De Meyer M, Backeljau T, Gombeer S, Meganck K, Vanderheyden A, Müller R,

Van Bortel W (2022a) MEMO: Monitoring of exotic mosquitoes in Belgium. Gigabyte, 1–9. https://doi.org/10.46471/gigabyte.59

Deblauwe I, De Wolf K, De Witte J, Schneider A, Verle I, Vanslembrouck A, Smitz N, Demeulemeester J, Van Loo T, Dekoninck W, Krit M, Madder M, Müller R, Van Bortel W (2022b) From a long-distance threat to the invasion front: A review of the invasive *Aedes* mosquito species in Belgium between 2007 and 2020. Parasites & Vectors 15(1): 206. https://doi.org/10.1186/s13071-022-05303-w

Delnat V, Janssens L, Stoks R (2020) Effects of predator cues and pesticide resistance on the toxicity of a (bio)pesticide mixture. Pest Management Science 76(4): 1448–1455. https://doi.org/10.1002/ps.5658

Dobson SL (2021) When more is less: Mosquito population suppression using sterile, incompatible and genetically modified male mosquitoes. Journal of Medical Entomology 58(5): 1980–1986. https://doi.org/10.1093/jme/tjab025

Donald CL, Siriyasatien P, Kohl A (2020) Toxorhynchites species: A review of current knowledge. Insects 11(11): 747. https://doi.org/10.3390/insects11110747

Eyre MD, Ball SG, Foster GN (1986) An initial classification of the habitats of aquatic Coleoptera in North-East England. Journal of Applied Ecology 23(3): 841–852. https://doi.org/10.2307/2403938

Farajollahi A, Price DC (2013) A rapid identification guide for larvae of the most common North American container-inhabiting *Aedes* species of medical importance. Journal of the American Mosquito Control Association 29(3): 203–221. https://doi.org/10.2987/11-6198R.1

Flandes X, Hansen CA, Palani S, Abbas K, Bennett C, Caro WP, Hutubessy R, Khazhidinov K, Lambach P, Maure C, Marshall C, Rojas DP, Rosewell A, Sahastrabuddhe S, Tufet M, Wilder-Smith A, Beasley DWC, Bourne N, Barrett ADT (2023) Vaccine value profile for Chikungunya. Vaccine 42(19): S9–S24. https://doi.org/10.1016/j.vaccine.2023.07.069

Foster GN, Friday LE (2011) Keys to the water beetles of Britain and Ireland (part1). Field Studies Council, Shrewsbury, 152 pp. https://doi.org/10.1079/9781800625730.0000

Fransiscolo ME (1979) Coleoptera Haliplidae, Hygrobiidae, Gyrinidae, Dytiscidae. Fauna d'Italia, Bologna.

Galewski K (1971) A study on morphobiotic adaptations of European species of the Dytiscidae (Coleoptera). Polskie Pismo Entomologiczne 41(3): 479–702.

Galewski K (1973) Stadial differentiation in larvae of the Dytiscidae (Coleoptera). Polskie Pismo Entomologiczne 43: 15–25.

Giunti G, Becker N, Benelli G (2023) Invasive mosquito vectors in Europe: From bioecology to surveillance and management. Acta Tropica 239: 106832. https://doi.org/10.1016/j.actatropica.2023.106832

Gossner CM, Ducheyne E, Schaffner F (2018) Increased risk for autochthonous vector-borne infections transmitted by *Aedes albopictus* in continental Europe. Eurosurveillance 23(24). https://doi.org/10.2807/1560-7917.ES.2018.23.24.1800268

Graziosi I, Aranda C, Balestrino F, Bellini R, Busquets N, Coulibaly M, Crisanti A, Diallo D, Diallo M, Gaye A, Guelbeogo M, Ignjatović-Ćupina A, Napp S, N'Falé S, Petrić D, Pollegioni P, Simoni A, Zgomba M, Müller R (2020) A litmus test for harmonized mosquito monitoring across Europe and Africa. InfraVec2 Output manuscript, 22 pp. https://doi.org/10.1101/2020.01.30.927020

Inoda T, Kamimura S (2004) New open aquarium system to breed larvae of water beetles (Coleoptera: Dytiscidae). Coleopterists Bulletin 58(1): 37–43. https://doi.org/10.1649/591

Inoda T, Kitano T (2013) Mass breeding larvae of the critically endangered diving beetles Dytiscus sharpi sharpi and Dytiscus sharpi validus (Coleoptera: Dytiscidae). Applied Entomology and Zoology 48(3): 397–401. https://doi.org/10.1007/s13355-013-0176-4

Jaffal A, Fite J, Baldet T, Delaunay P, Jourdain F, Mora-Castillo R, Olive MM, Roiz D (2023) Current evidences of the efficacy of mosquito mass-trapping interventions to reduce *Aedes aegypti*

and *Aedes albopictus* populations and *Aedes*-borne virus transmission. PLoS Neglected Tropical Diseases 17(3): e0011153. https://doi.org/10.1371/journal.pntd.0011153

James HG (1965) Predators of *Aedes atropalpus* (Diptera: Culicidae) and of other moquitoes breeding in rock pools in Ontario. Canadian Journal of Zoology 43(1): 5. https://doi.org/10.1139/z65-013

Jones RT, Ant TH, Cameron MM, Logan JG (2021) Novel control strategies for mosquito-borne diseases. Philosophical Transactions of the Royal Society B 376(1818): 20190802. https://doi.org/10.1098/rstb.2019.0802

Jourdan J, Riesch R, Cunze S (2021) Off to new shores: Climate niche expansion in invasive mosquitofish (*Gambusia* spp.). Ecology and Evolution 11(24): 18369–18400. https://doi.org/10.1002/ece3.8427

Juliano SA, Lounibos LP (2005) Ecology of invasive mosquitoes: Effects on resident species and on human health. Ecology Letters 8(5): 558–574. https://doi.org/10.1111/j.1461-0248.2005.00755.x

Kehl S, Dettner K (2007) Flugfähigkeit der in Deutschland vorkommenden adephagen Wasserkäfer (Coleoptera, Hydradephaga). Entmologie heute (19): 141–161.

Kesavaraju B, Brey CW, Farajollahi A, Evans HL, Gaugler R (2011) Effect of malathion on larval competition between *Aedes albopictus* and *Aedes atropalpus* (Diptera: Culicidae). Journal of Medical Entomology 48(2): 479–484. https://doi.org/10.1603/ME10101

Kitching RL, Orr AG (1996) The foodweb from water-filled treeholes in Kuala Belalong, Brunei. The Raffles Bulletin of Zoology 44(2): 405–413.

Kramer IM, Kress A, Klingelhofer D, Scherer C, Phuyal P, Kuch U, Ahrens B, Groneberg DA, Dhimal M, Muller R (2020) Does winter cold really limit the dengue vector *Aedes aegypti* in Europe? Parasites & Vectors 13(1): 178. https://doi.org/10.1186/s13071-020-04054-w

Kramer IM, Pfeiffer M, Steffens O, Schneider F, Gerger V, Phuyal P, Braun M, Magdeburg A, Ahrens B, Groneberg DA, Kuch U, Dhimal M, Muller R (2021) The ecophysiological plasticity of *Aedes aegypti* and *Aedes albopictus* concerning overwintering in cooler ecoregions is driven by local climate and acclimation capacity. The Science of the Total Environment 778: 146128. https://doi.org/10.1016/j.scitotenv.2021.146128

Kroeger I, Liess M, Dziock F, Duquesne S (2013) Sustainable control of mosquito larvae in the field by the combined actions of the biological insecticide Bti and natural competitors. Journal of Vector Ecology 38(1): 82–89. https://doi.org/10.1111/j.1948-7134.2013.12012.x

Kumar NP, Bashir A, Abidha S, Sabesan S, Jambulingam P (2014) Predatory potential of *Platynectes* sp. (Coleoptera: Dytiscidae) on *Aedes albopictus*, the vector of dengue/chikungunya in Kerala, India. Tropical Biomedicine 31(4): 736–741.

Larson DJ, Alarie Y, Roughley RE (2000) Predacious diving beetles (Coleoptera: Dytiscidae) of the Nearctic region. NRC Research Press, Ottawa.

Liao W, Venn S, Niemelä J (2020) Environmental determinants of diving beetle assemblages (Coleoptera: Dytiscidae) in an urban landscape. Biodiversity and Conservation 29(7): 2343–2359. https://doi.org/10.1007/s10531-020-01977-9

Lundkvist E, Landin J, Karlsson F (2002) Dispersing diving beetles (Dytiscidae) in agricultural and urban landscapes in south-eastern Sweden. Annales Zoologici Fennici (39): 109–123.

Lundkvist E, Landin J, Jackson M, Svensson C (2003) Diving beetles (Dytiscidae) as predators of mosquito larvae (Culicidae) in field experiments and in laboratory tests of prey preference. Bulletin of Entomological Research 93(3): 219–226. https://doi.org/10.1079/BER2003237

Malla RK, Mandal KK, Burman S, Das S, Ghosh A, Chandra G (2023) Numerical analysis of predatory potentiality of *Toxorhynchites splendens* against larval *Aedes albopictus* in laboratory and semi-field conditions. Scientific Reports 13(1): 7403. https://doi.org/10.1038/s41598-023-34651-5

Martinet JP, Ferte H, Failloux AB, Schaffner F, Depaquit J (2019) Mosquitoes of North-Western Europe as Potential Vectors of Arboviruses: A Review. Viruses 11(11): 1059. https://doi.org/10.3390/v11111059

Miller KB, Bergsten J (2016) Diving beetles of the world. Johns Hopkins University Press, Baltimore, Maryland. https://doi.org/10.1353/book.48093

Müller R, Knautz T, Vollroth S, Berger R, Kress A, Reuss F, Groneberg DA, Kuch U (2018) Larval superiority of *Culex pipiens* to *Aedes albopictus* in a replacement series experiment: Prospects for coexistence in Germany. Parasites & Vectors 11(1): 80. https://doi.org/10.1186/s13071-018-2665-3

Nilsson AN (2011) A world catalogue of the family Noteridae, or the burrowing water beetles (Coleoptera, Adephaga) (16/08/2011 ed.). University of Umeå, Sweden.

Nilsson AN (2024) A paddling pool, an aquarium and three bippos. Latissimus 56: 1–4.

Nilsson AN, Hájek J (2024) Catalogue of Palearctic Dytiscidae (Coleoptera). (Vol. 3). Apollo books, Stenstrup.

Nilsson AN, Holmen M (1995) The aquatic Adephaga (Coleoptera) of Fennoscandia and Denmark. II. Dytiscidae (Vol. 32). Fauna Entomologica Scandinavia, Leiden, New York, Köln. https://doi.org/10.1163/9789004273603

Ogunlade ST, Meehan MT, Adekunle AI, McBryde ES (2023) A Systematic Review of Mathematical Models of Dengue Transmission and Vector Control: 2010–2020. Viruses 15(1): 254. https://doi.org/10.3390/v15010254

Ohba SY, Takagi M (2010) Predatory Ability of Adult Diving Beetles on the Japanese Encephalitis Vector *Culex tritaeniorhynchus.* Journal of the American Mosquito Control Association 26(1): 32–36. https://doi.org/10.2987/09-5946.1

Ohba SY, Ushio M (2015) Effect of water depth on predation frequency by diving beetles on mosquito larvae prey. Entomological Science 18(4): 519–522. https://doi.org/10.1111/ens.12143

Onyeka JOA (1983) Studies on the Natural Predators of *Culex pipiens* L and *Culex torrentium* Martini (Diptera, Culicidae) in England. Bulletin of Entomological Research 73(2): 185–194. https://doi.org/10.1017/S0007485300008798

Op De Beeck L, Janssens L, Stoks R (2016) Synthetic predator cues impair immune function and make the biological pesticide Bti more lethal for vector mosquitoes. Ecological Applications 26(2): 355–366.

Pauly I, Jakoby O, Becker N (2022) Efficacy of native cyclopoid copepods in biological vector control with regard to their predatory behavior against the Asian tiger mosquito, *Aedes albopictus.* Parasites & Vectors 15(1): 351. https://doi.org/10.1186/s13071-022-05460-y

Perrin A, Glaizot O, Christe P (2022) Worldwide impacts of landscape anthropization on mosquito abundance and diversity: A meta-analysis. Global Change Biology 28(23): 6857–6871. https://doi.org/10.1111/gcb.16406

Pichler V, Caputo B, Valadas V, Micocci M, Horvath C, Virgillito C, Akiner M, Balatsos G, Bender C, Besnard G, Bravo-Barriga D, Bueno-Mari R, Collantes F, Delacour-Estrella S, Dikolli E, Falcuta E, Flacio E, Garcia-Perez AL, Kalan K, Kavran M, L'Ambert G, Lia RP, Marabuto E, Medialdea R, Melero-Alcibar R, Michaelakis A, Mihalca A, Mikov O, Miranda MA, Muller P, Otranto D, Pajovic I, Petric D, Rebelo MT, Robert V, Rogozi E, Tello A, Zitko T, Schaffner F, Pinto J, Della Torre A (2022) Geographic distribution of the V1016G knockdown resistance mutation in *Aedes albopictus*: A warning bell for Europe. Parasites & Vectors 15(1): 280. https://doi.org/10.1186/s13071-022-05407-3

Pleydell DRJ, Bouyer J (2019) Biopesticides improve efficiency of the sterile insect technique for controlling mosquito-driven dengue epidemics. Communications Biology 2(1): 201. https://doi.org/10.1038/s42003-019-0451-1

Reuss F, Kress A, Braun M, Magdeburg A, Pfenninger M, Muller R, Mehring M (2020) Knowledge on exotic mosquitoes in Germany, and public acceptance and effectiveness of Bti and two self-prepared insecticides against *Aedes japonicus japonicus.* Scientific Reports 10(1): 18901. https://doi.org/10.1038/s41598-020-75780-5

Reyne M, Nolan M, McGuiggan H, Aubry A, Emmerson M, Marnell F, Reid N (2020) Artificial agri-environment scheme ponds do not replicate natural environments despite higher aquatic and terrestrial invertebrate richness and abundance. Journal of Applied Ecology 58(2): 281–285. https://doi.org/10.1111/1365-2664.13738

Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR (2019) Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. PLoS Neglected Tropical Diseases 13(3): e0007213. https://doi.org/10.1371/journal.pntd.0007213

Scheers K, Dopagne C, Thys N (in press) An annotated checklist of the Hydradephaga of Belgium (Coleoptera: Dytiscidae; Gyrinidae; Haliplidae, Hygrobiidae & Noteridae). Belgian Journal of Entomology.

Schindler S, Staska B, Adam M, Rabitsch W, Essl F (2015) Alien species and public health impacts in Europe: A literature review. NeoBiota 27: 1–23. https://doi.org/10.3897/neobiota.27.5007

Schmalenbach I, Buchholz F, Franke HD, Saborowski R (2009) Improvement of rearing conditions for juvenile lobsters (*Homarus gammarus*) by co-culturing with juvenile isopods (*Idotea emarginata*). Aquaculture (Amsterdam, Netherlands) 289(3–4): 297–303. https://doi.org/10.1016/j.aquaculture.2009.01.017

Senapati A, Sardar T, Ganguly KS, Ganguly KS, Chattopadhyay AK, Chattopadhyay J (2019) Impact of adult mosquito control on dengue prevalence in a multi-patch setting: A case study in Kolkata (2014–2015). Journal of Theoretical Biology 478: 139–152. https://doi.org/10.1016/j.jtbi.2019.06.021

Service MW (1977) Ecological and Biological Studies on *Aedes cantans* (Meig) (Diptera-Culicidae) in Southern England. Journal of Applied Ecology 14(1): 159–196. https://doi.org/10.2307/2401833

Shaalan EA, Canyon DV (2009) Aquatic insect predators and mosquito control. Tropical Biomedicine 26(3): 223–261. https://www.ncbi.nlm.nih.gov/pubmed/20237438

Shaverdo HV, Esfandiari M, Khadempur A, Nasserzadeh H, Ghodrati A (2013) Diving beetles of Ahvaz City, Khuzestan Province, Iran (Coleoptera: Dytiscidae). Koleopterologische Rundschau 83: 17–22.

Sherpa S, Blum MGB, Capblancq T, Cumer T, Rioux D, Despres L (2019) Unravelling the invasion history of the Asian tiger mosquito in Europe. Molecular Ecology 28(9): 2360–2377. https://doi.org/10.1111/mec.15071

Shine R, Ward-Fear G, Brown GP (2020) A famous failure: Why were cane toads an ineffective biocontrol in Australia? Conservation Science and Practice 2(12): e296. https://doi.org/10.1111/csp2.296

Skuse FAA (1894) The banded mosquito of Bengal. Indian Museum Notes 3(5): 20.

Smitz N, De Wolf K, Deblauwe I, Kampen H, Schaffner F, De Witte J, Schneider A, Verle I, Vanslembrouck A, Dekoninck W, Meganck K, Gombeer S, Vanderheyden A, De Meyer M, Backeljau T, Werner D, Müller R, Van Bortel W (2021) Population genetic structure of the Asian bush mosquito, *Aedes japonicus* (Diptera, Culicidae), in Belgium suggests multiple introductions. Parasites & Vectors 14(1): 179. https://doi.org/10.1186/s13071-021-04676-8

Su X, Guo Y, Deng J, Xu J, Zhou G, Zhou T, Li Y, Zhong D, Kong L, Wang X, Liu M, Wu K, Yan G, Chen XG (2019) Fast emerging insecticide resistance in *Aedes albopictus* in Guangzhou, China: Alarm to the dengue epidemic. PLoS Neglected Tropical Diseases 13(9): e0007665. https://doi.org/10.1371/journal.pntd.0007665

Sulaiman S, Jeffery J (1986) The ecology of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in a rubber estate in Malaysia. Bull. ent. Bulletin of Entomological Research 76(4): 553–557. https://doi.org/10.1017/S0007485300015066

Thomas SJ (2023) Is new dengue vaccine efficacy data a relief or cause for concern? NPJ Vaccines 8(1): 55. https://doi.org/10.1038/s41541-023-00658-2

Van Handel E (1985a) Rapid determination of glycogen and sugars in mosquitoes. Journal of the American Mosquito Control Association 1(3): 299–301. https://www.ncbi.nlm.nih.gov/pubmed/2906671

Van Handel E (1985b) Rapid determination of total lipids in mosquitoes. Journal of the American Mosquito Control Association 1(3): 302–304. https://www.ncbi.nlm.nih.gov/pubmed/2906672

Vereecken S, Vanslembrouck A, Kramer IM, Müller R (2022) Phenotypic insecticide resistance status of the *Culex pipiens* complex: A European perspective. Parasites & Vectors 15(1): 423. https://doi.org/10.1186/s13071-022-05542-x

von der Leyen (2022) Amending Implementing Regulation (EU) 2016/1141 to update the list of invasive alien species of Union concern L 186/10 C.F.R. (2022).

Vontas J, Kioulos E, Pavlidi N, Morou E, della Torre A, Ranson H (2012) Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti.* Pesticide Biochemistry and Physiology 104(2): 126–131. https://doi.org/10.1016/j.pestbp.2012.05.008

Yanoviak SP (2001) The macrofauna of water-filled tree holes on Barro Colorado Island, Panama. Biotropica 33(1): 110–120. https://doi.org/10.1111/j.1744-7429.2001.tb00161.x

Yee DA (2014) Ecology, systematics, and the natural history of predacious diving beetles (Coleoptera: Dytiscidae). Springer, Dordrecht, Heidelberg, New York, London. https://doi.org/10.1007/978-94-017-9109-0

Yee DA, Kesavaraju B, Juliano SA (2004) Larval feeding behavior of three co-occurring species of container mosquitoes. Journal of Vector Ecology 29(2): 315–322. https://www.ncbi.nlm.nih.gov/pubmed/15707290

Younes AA (2008) Predation of the diving beetle, *Eretes sticticus* (Coleoptera: Dytiscidae) on mosquito larvae, *Culex pipiens* L. (Diptera: Culicidae). Egyptian Journal of Biological Pest Control (18(2)): 303–308.

Young FN (1954) The water beetles of Florida. (Vol. 5): University of Florida Studies.

Supplementary material 1

More information on the aquatic beetle taxa that were collected and used during the experiments

Authors: Adwine Vanslembrouck, Kevin Scheers, Xavier Vermeersch, Rens Hendrickx, Anna Schneider, Jacobus De Witte, Isra Deblauwe, Wim Van Bortel, Friederike Reuss, Ruth Müller

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.94.121987.suppl1

Supplementary material 2

General overview of larval benthic feeding behaviour of *A. albopictus* (followed) and filter feeding behaviour at water surface of *C. pipiens*

Authors: Adwine Vanslembrouck, Kevin Scheers, Xavier Vermeersch, Rens Hendrickx, Anna Schneider, Jacobus De Witte, Isra Deblauwe, Wim Van Bortel, Friederike Reuss, Ruth Müller

Data type: mp4

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.94.121987.suppl2

Supplementary material 3

The benthic feeding behaviour of *A. albopictus* larvae

Authors: Adwine Vanslembrouck, Kevin Scheers, Xavier Vermeersch, Rens Hendrickx, Anna Schneider, Jacobus De Witte, Isra Deblauwe, Wim Van Bortel, Friederike Reuss, Ruth Müller

Data type: mp4

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.94.121987.suppl3

Supplementary material 4

Filter feeding behaviour of *C. pipiens* at the water surface

Authors: Adwine Vanslembrouck, Kevin Scheers, Xavier Vermeersch, Rens Hendrickx, Anna Schneider, Jacobus De Witte, Isra Deblauwe, Wim Van Bortel, Friederike Reuss, Ruth Müller

Data type: mp4

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.94.121987.suppl4

Supplementary material 5

Predation of *Laccophilus minutus* on *Aedes albopictus* larvae

Authors: Adwine Vanslembrouck, Kevin Scheers, Xavier Vermeersch, Rens Hendrickx, Anna Schneider, Jacobus De Witte, Isra Deblauwe, Wim Van Bortel, Friederike Reuss, Ruth Müller

Data type: mp4

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/neobiota.94.121987.suppl5